

MUSCULAR ACTIVITY UNDERLYING VENTILATION AND
SWIMMING IN THE HORSESHOE CRAB,
LIMULUS POLYPHEMUS (LINNAEUS)¹

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Ventilation and swimming in *Limulus polyphemus* (Linnaeus) are two behaviorally distinct, rhythmic movements involving coordinated metachronal activation of the opisthosomal appendages. Normally, ventilation consists of slow, rhythmic movements of all five gill plate pairs causing circulation of water through the subjacent gill books. The ventilatory cycle ranges from a low frequency, low amplitude movement of only the terminal gill plates to a vigorous rhythm (hyperventilation) involving the genital operculum as well as all gill plates. Vigorous ventilatory movements, however, do not develop significant propulsive force. Swimming consists of rapid gill plate and opercular movements which are qualitatively similar to those of ventilation but which differ substantially in amplitude and various temporal characteristics. Frequently the prosomal walking legs participate in swimming behavior, moving in coordination with the opisthosomal appendages.

Hyde (1893) found that *Limulus* opisthosomal ganglia, isolated from the rest of the CNS by nerve sectioning, are capable of generating ventilatory movements in their corresponding gill plates. More recently Fournier, Drewes and Pax (1971) demonstrated that the motor output is sustained in the absence of peripheral feedback and therefore probably is centrally programmed. Intracellular recordings and stimulation revealed that motoneurons participating in the ventilatory bursts do not interact (Wyse, 1971). These results suggest that the origin of the oscillatory motor output which controls ventilation involves premotor interneurons within each opisthosomal ganglion. The overt similarities between swimming and ventilatory movements, and the fact that reciprocal excitation of the same antagonistic flexor and extensor muscles are responsible for the gill plate movements of both behaviors, suggest a common central control mechanism of rhythmic output. This investigation utilizes gill plate muscle activity recorded during periods of swimming and ventilation to predict characteristics of the underlying motoneuron bursts and to analyze various aspects of their coordinating mechanisms.

MATERIAL AND METHODS

Horseshoe crabs, *Limulus polyphemus*, were collected by the supply department of the Marine Biological Laboratory (Woods Hole, Massachusetts) and held

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until use in tanks of running sea water at room temperature either in Woods Hole or in Santa Barbara. A Nikon Super 8 movie camera (film speed 24 frames per second) was used for detailed observation of appendage movements during *Limulus* swimming and ventilation. The films were analyzed by frame-by-frame projection and tracing of the position of each participating appendage as it underwent metachronal movements. The paths described by the basal segment of each leg and the distal articulation of the opisthosomal appendages were plotted as a function of time.

Ventilation was monitored while the animal rested submerged in a container of sea water. Swimming studies were conducted with the animal suspended by a clamp on the prosomal carapace, which allowed free movement of the opisthosoma and all appendages. Specimens supported in this manner usually began swimming spontaneously, although in some cases gentle tactile stimulation was required to elicit this behavior.

Electromyograms were recorded from the prosomal tergo-coxal muscles, which retract the ambulatory appendages, and the opisthosomal abductor and external branchial muscles, which extend and flex respectively the gill plates and the genital operculum (for terminology of the musculature see Lankerster, Benham, and Beck, 1885). Monopolar electrodes of insulated silver or copper wire were implanted through small holes bored in the prosomal or opisthosomal carapace at the tergal insertion of the appropriate muscles. The electrodes were secured to the carapace with Eastman 9-10 adhesive and plastic tape. Muscle potentials were amplified with Tektronix Type 122 low-level preamplifiers and displayed on a Tektronix 564 oscilloscope or an Offner Type RB dynograph. A one second time constant was used for integrated records of muscle activity.

Since variations in the degree of electrical contact made by individual electrodes bias the recorded muscle activity amplitudes and durations, only burst interval data are quantitatively comparable between electrodes. Comparisons between electromyogram amplitude and duration are valid only if the data were recorded from the same electrode implantation. Thus, burst amplitude is here computed as the percentage of maximum amplitude recorded during rapid swimming movements, and intersegmental delay is defined as the measured time between the peaks of integrated burst activity from sequential extensor or flexor muscles.

RESULTS

Ventilation

Ventilation consists of repetitive extensions and flexions of the opisthosomal gill plates beginning with the terminal pair and proceeding sequentially forward. Each gill plate extends slowly, actively bent at its distal articulation by muscles in the outer lobe, and sweeps out an arc from approximately 3-5 degrees during slow ventilation to 65-70 degrees during periods of hyperventilation. Extension of the gill plate forces the branchial leaflets apart, allowing for the irrigation of the subjacent gill books. Gill plate extensions are followed by a metachronal wave of more rapid gill plate flexions, during which time the outer lobe muscles of each plate momentarily relax. Each gill plate is extended in response to a burst of activity in its corresponding abductor muscle (Fig. 1a, 1b). The burst is almost symmetrical in shape with the amplitude peak toward the center of the burst. Gill plate flexion results from a similarly symmetrical muscle burst in the external

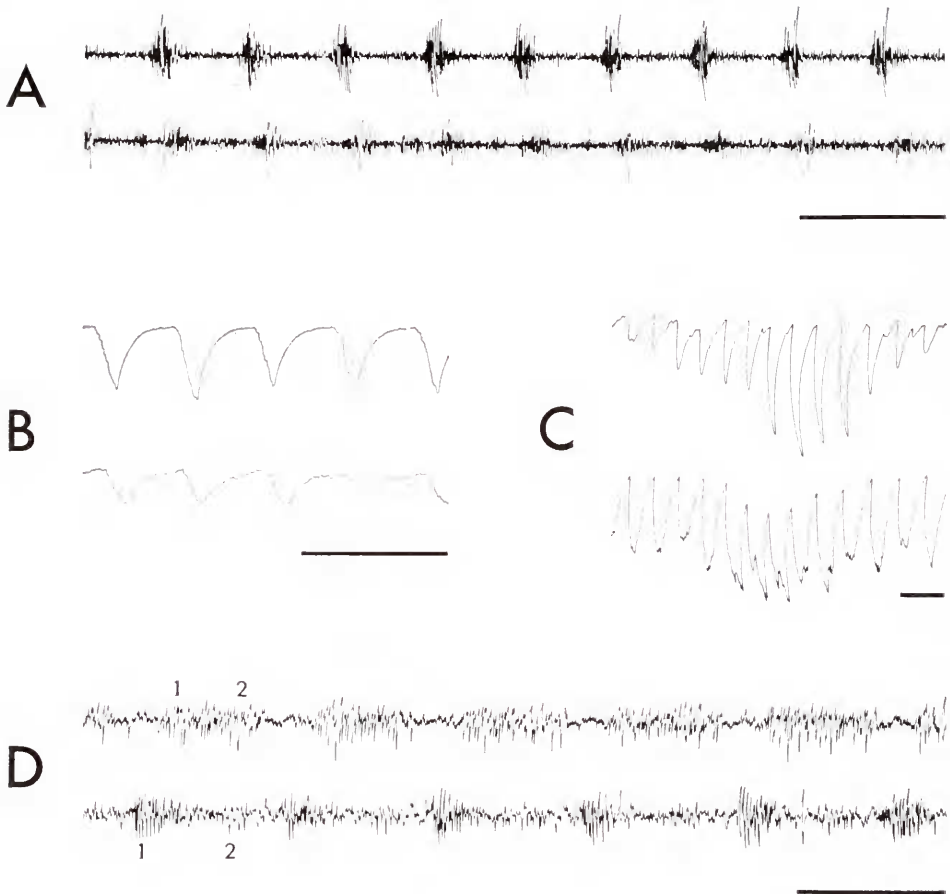


FIGURE 1. Flexor and extensor bursts during ventilation and hyperventilation. (A) exemplifies the reciprocal activity in flexor (top) and extensor (bottom) muscles during ventilation as recorded from the musculature of the first gill plate. (B) is an integrated record of (A) showing the symmetrical shape of the flexor and extensor muscle bursts (a downward deflection indicates activity). In (C) an integrated record of flexor (top) and extensor (bottom) bursts during gradual transitions from ventilation to hyperventilation and back to ventilation demonstrate the concomitant transformations of the flexor burst shape from symmetrical to saw-tooth, and of the extensor burst shape from symmetrical to double peaked. (D) shows double peaked extensor bursts in the first (top) and fourth (bottom) gill plates during hyperventilation. The first activity peak (1) in both muscles is consistently strong, whereas the second activity peak (2) is considerably weaker in the more caudal muscle. Bar indicates 5 sec.

branchial muscle (Fig. 1b). These muscle burst shapes correlate with the symmetrical motoneuron firing patterns in the external branchial nerve and medial branchial nerve as recorded by Fournier *et al.* (1971).

Electromyograms from extensor and flexor muscles indicate that the following elements of the ventilatory burst cycle are variable: (1) the number of participating appendages; (2) the muscle burst interval; (3) the intersegmental delay of muscle

TABLE I

Recorded ranges of the variable elements in flexor muscle burst during ventilation, hyperventilation and swimming (Values taken from data on sixteen different animals). Abbreviations are: gp, gill plate; go, genital operculum; wl, walking leg.

	Appendages involved		Burst interval (sec)		Burst duration (sec)		Burst amplitude (per cent max.)		Intersegmental delay*** (msec)	
	Slow*	Fast**	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
Ventilation	gp 1	gp 1-5	20.0	1.0	1.80	0.28	<5%	40%	500	30
Hyperventilation	gp 1-5	gp 1-5	4.0	0.6	1.00	0.25	30%	85%	70	10
Swimming	go	go	1.8	0.4	1.10	0.20	78%	100%	30	<10
	gp 1-5	gp 1-5								
	go	go wl 1-5								

* slow limits were set by larger animals (16 to 18 cm across the prosomal carapace).

** fast limits were set by smaller animals (8 to 10 cm across the prosomal carapace).

*** the measured time between peaks of integrated burst activity from sequential flexor muscles.

activation; (4) the amplitude of integrated muscle activity; and (5) the duration of the muscle activity. The recorded range for each element of the flexor burst cycle is presented in Table I. Occasionally, during periods of extremely slow ventilation, only the caudal gill plate pair or pairs are active; these movements are slight, with correspondingly small muscle potentials and large intersegmental delays, up to 500 msec in some cases. If the animal receives gentle tactile stimulation, the more rostral gill plates and the genital operculum will be recruited into the rhythmic movement with a concomitant increase in both cycle frequency and muscle activity, and a decrease in the intersegmental delay. Figure 2 suggests that the above variables are linearly related to burst interval.

Hyperventilation

Hyperventilation is behaviorally distinguishable from ventilation by the active participation of the genital operculum in the rhythmic metachronal cycle. Since there are no gill books associated with the operculum, this movement appears only to enhance the circulation of water for the more caudal gill plates and their corresponding gill books. The transition from ventilation to hyperventilation may be gradual, with no obvious change in the metachronal movements other than incorporation of the genital operculum, or it may be abrupt, in which case there will be observed a pronounced increase in amplitude of the gill plate movements (Fig. 3). As hyperventilation becomes more vigorous, a transition in the extensor and flexor muscle burst shapes takes place (Fig. 1c). The extensor burst assumes a double peak conformation in which the first peak is larger and more consistent, while the second is somewhat erratic, being almost imperceptible in the most caudal gill plates, and becoming larger in the more rostral gill plates (Fig. 1d). The burst shape in the flexor muscle changes from symmetrical, during slow to moderate hyperventilation, to a saw-tooth shape during rapid hyperventilation (Fig. 1c). This transition may occur gradually, in which case the peak of muscle activity appears to migrate slowly toward the beginning of the burst. This change in

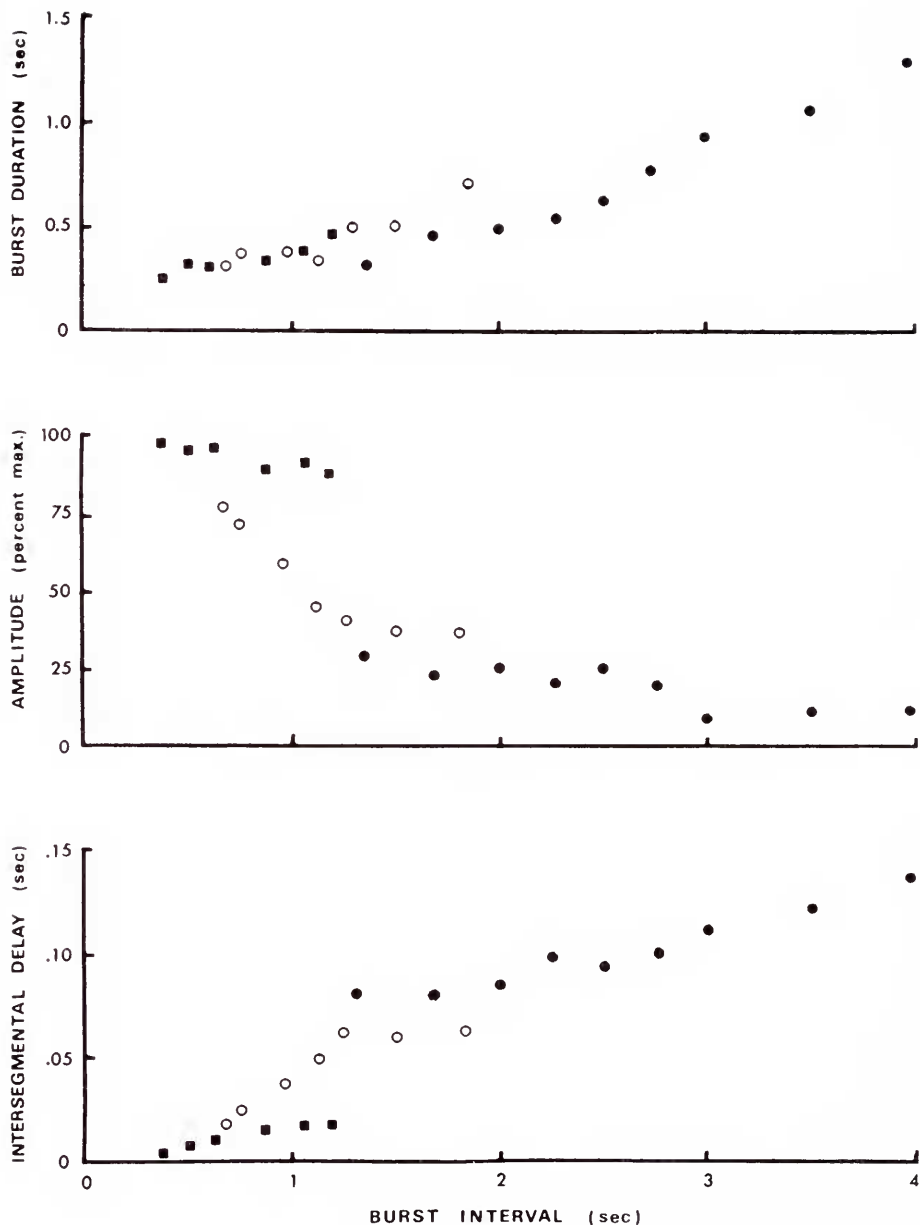


FIGURE 2. Change in flexor burst duration, amplitude and intersegmental delay as a function of muscle burst interval for ventilation (closed circles), hyperventilation (open circles), and swimming (closed squares). The data was taken from the flexor muscles in the first and second gill plates of 12 cm (across prosoma) males ($N = 10$). Similar relationships are found in the extensor muscle activity.

shape reflects a similar alteration in the causative motoneuron burst from one which is symmetrical to one which is biased toward burst onset.

Measurements of hyperventilatory intersegmental delay and muscle potential amplitude vary from those recorded during ventilatory movements (Fig. 2). Flexor and extensor muscles undergo substantially greater increases in muscle amplitude and decreases in intersegmental delay with decreasing burst interval. Also, the lower limit of the burst interval greatly decreases during hyperventilation (Table I), which allows for more rapid movements of the gill plates than occurs during ventilation.

Swimming

Swimming behavior varies from the previously described activities of ventilation and hyperventilation in that prosomal appendages are incorporated into the ongoing metachronal process. The onset of swimming is signaled by a slight retraction of the fifth pair of walking legs, which usually does not affect the anterior appendages. This retraction is immediately followed by repetitive waves of coordinated appendage protractions and retractions beginning at the terminal gill plates and proceeding rostrally to the first pair of walking legs (Fig. 4). The gill plates move powerfully following the basic ventilatory movement pattern, but sweep out a considerably greater arc and bend to a greater extent at their distal articulation during extension than is the case for ventilation. The prosomal walking legs, on the other hand, describe a more elliptical path (as viewed from the side) (Fig. 5), protracting slowly under the protection of the carapace and then suddenly extending and rapidly retracting, beginning with the fifth leg. Movement of the fifth leg is followed by a simultaneous retraction of the four anterior legs. The chelae of the first four walking legs, and the spatulate spines of the fifth walking leg spread simultaneously with extension which probably serves to increase thrust. Following retraction, the chelae and tarsal spines close and the legs are again brought up under the carapace.

All the aforementioned appendages continue in their stereotyped movements as long as the specimen swims unobstructedly. If, however, an obstacle is contacted,

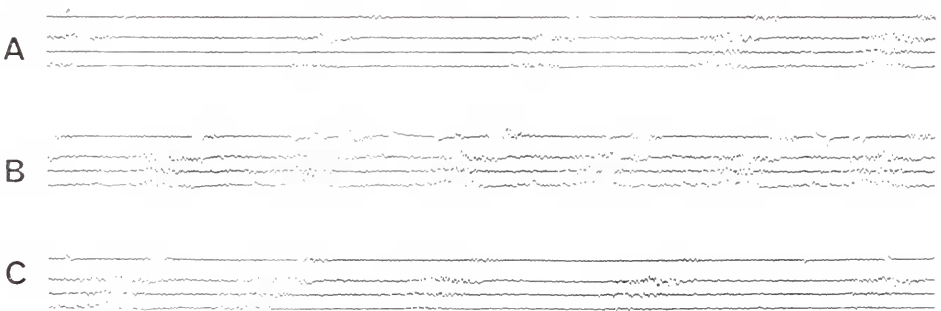


FIGURE 3. Activation and deactivation of the flexor muscle to the genital operculum during a transition from ventilation (A) to hyperventilation (B) and back to ventilation (C). Shown are consecutive recordings from the flexor muscles of the genital operculum and first, second and third gill plates (top to bottom). Bar indicates 0.5 sec.

the animal can navigate around it by maintaining swimming movements in certain legs while others remain at rest. Thus all the legs have been observed operating independently of the others and in various combinations with ipsilateral as well as contralateral legs, and independently of the gill plates and the operculum. The opisthosomal appendages, however, do not exhibit this degree of freedom. Every wave initiated by the fifth gill plate during swimming carries through to the operculum, which is not the case for ventilation where occasionally only the posterior two or three gill plate pairs will be affected by a single wave of excitation.

It is possible to identify swimming behavior from electromyograms since activity in the gill plate muscles is correlated with activity in the leg muscles. Analogous to the transition from ventilation to hyperventilation, the transition from ventilation to swimming may take place gradually, accompanied by a progressive increase in muscle burst amplitude and frequency (Fig. 6a), or suddenly, in which case burst amplitude and frequency increase abruptly (Fig. 6b). The extensor and flexor muscle bursts consist of uniform, high amplitude muscle potentials (Fig. 6a) which probably result from extremely intense short duration motor nerve activity. The temporal characteristics of the flexor and extensor bursts change little with decreasing burst interval during swimming, as opposed to the cases of ventilation and hyperventilation (Fig. 2). Although a discernible increase in burst amplitude and decrease in burst duration and intersegmental delay occurs with decreasing burst interval, the rates of change are less than those noted for hyperventilation and ventilation.

Lesion experiments

Lesion experiments similar to those by Hyde (1893) were performed in this study to segregate those opisthosomal movements which require contact with the brain from those which are within the repertoire of the isolated opisthosomal nervous system. A cut in the ventral nerve cord anywhere posterior to the brain leaves appendages caudal to the cut unable to carry out swimming movements, while appendages rostral to the cut perform apparently unaltered swimming motions. Appendages caudal to the cut will, however, participate in coordinated ventilation, which proceeds continually unless the gill plates are physically restrained, and hyperventilation, which may be provoked for short periods through tactile stimulation of the opisthosoma. Thus, the opisthosomal neural circuitry is capable of ventilatory and hyperventilatory movements in isolation, while swimming seems to require descending information from the brain.

Coordination in the ventral nerve cord

Tactile stimulation applied to the anterior lamella of any gill plate causes its immediate flexion and a general cessation of ventilatory movements, when the nervous system is intact. If, however, the ventral cord has been severed between the brain and first opisthosomal ganglion, this gill plate reflex is localized to the stimulated gill plate, which immediately resumes its ventilatory movements after flexing. To determine the extent to which such peripheral sensory input influences temporal aspects of the rhythmic activities, serial transections of the ventral nerve cord were made to isolate the fused fifth/fourth, the third, the second, and the first opisthosomal ganglia forcing them to coordinate their outputs through sensory

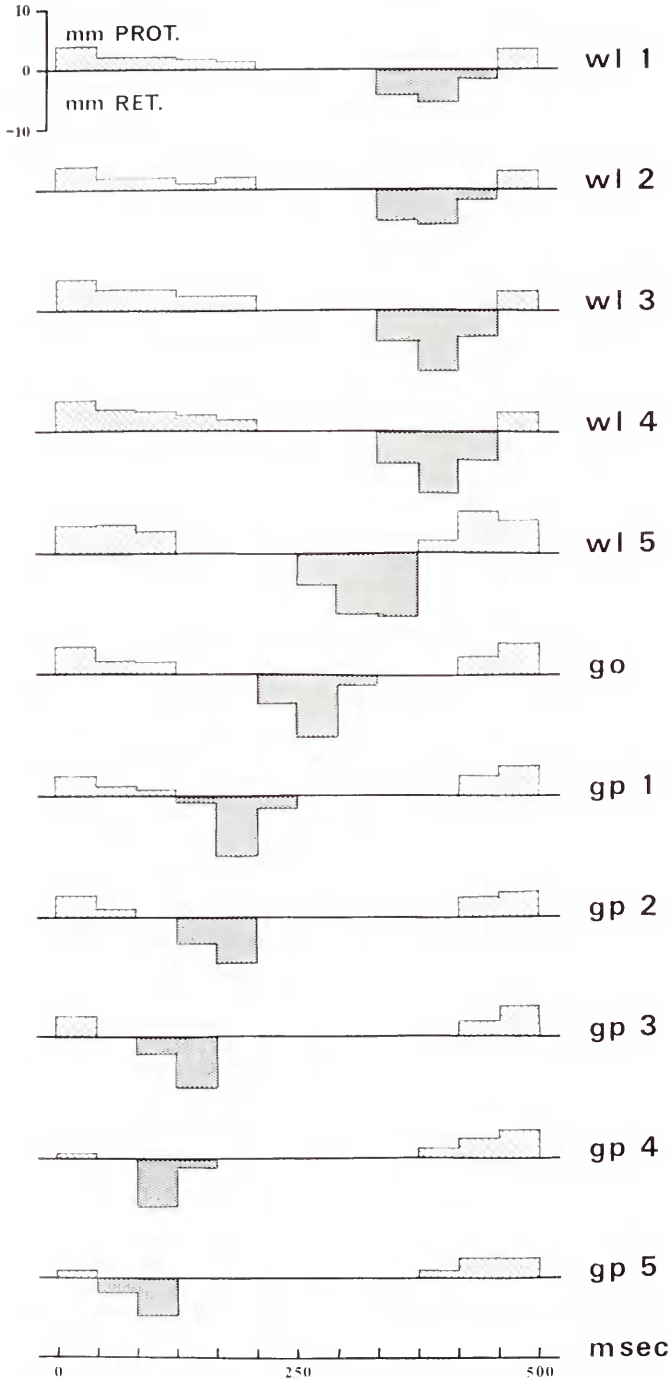


FIGURE 4. One swimming cycle of appendage protractions (positive) and retractions (negative) derived from frame by frame analysis of a freely swimming, 13 cm (prosomal

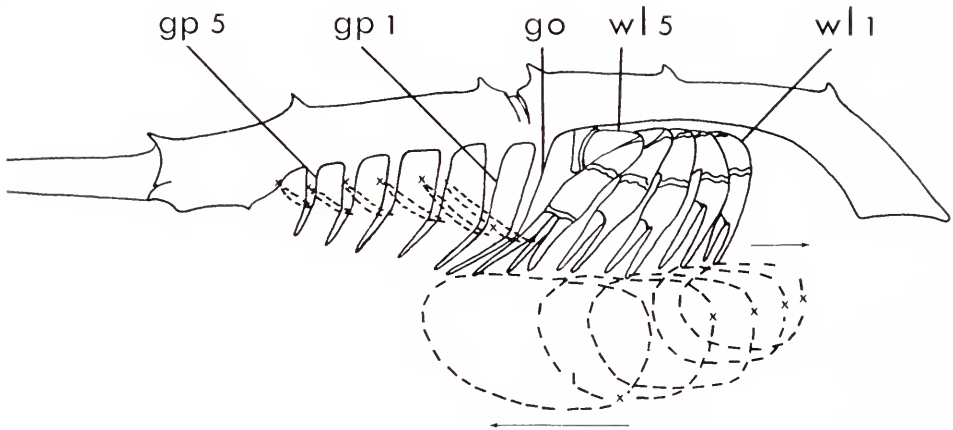


FIGURE 5. Cut-away view of *Limulus* showing the paths described by the various appendages during swimming as derived from consecutive frame tracings. The figure is drawn such that the appendage positions correspond to time 0 in Figure 4, and the "X's" denote the position of each appendage at 250 msec. Abbreviations are: go, genital operculum; gp, gill plate; wl, walking leg.

input alone. Muscle electrodes monitored activity in each external branchial muscle. Figure 7 shows the results of ganglionic isolation.

With each transection the following occurs: (1) there is a prolongation and sporadic desynchronization of the bursts from the isolated ganglion; (2) the pacemaker function is assumed by the most caudal intact ganglion; and (3) the burst duration and intersegmental delay of the intact ganglia become increasingly variable (Fig. 7b). All of the ganglia are capable of pacemaker activity and all maintain their rhythmic output after isolation. When all ganglia are isolated, the burst pattern of each remains loosely coordinated with activity in neighboring ganglia, although the order of burst initiation may vary (Fig. 7c). This loose coordination probably results from tactile stimulation by adjacent gill plates, whose movements trigger activity in other ganglia.

The pacemaker function, usually exercised by the fifth opisthosomal ganglion during normal ventilation, can be exercised by any of the first five ganglia under conditions of differential ganglionic stimulation if the nerve cord remains intact. Figure 8a represents the normal metachronal progression of the ventilatory rhythm. Figures 8b and 8c show the results of differential stimulation of the second and first ganglia by severing their gill plates. A distinct reversal in firing order is observed, with the stimulated ganglion assuming the pacemaker function for the system. Whichever ganglion functions as pacemaker, the coordinating circuitry is organized so that the wave of excitation propagates from the pacemaker, sequentially exciting adjacent ganglia both anteriorly and posteriorly.

width) female. Each bar represents the distance traveled, in a rostral or caudal direction, by the corresponding appendage between consecutive frames (41.7 msec). Abbreviations are: go, genital operculum; gp, gill plate; wl, walking leg (compare with Figure 5).

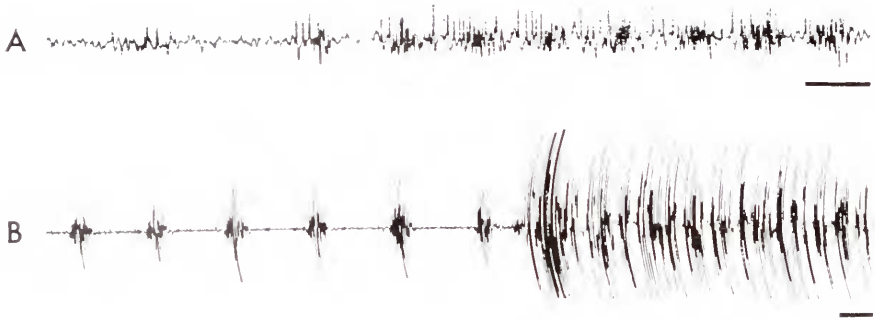


FIGURE 6. Flexor burst records during gradual (A) and abrupt (B) transitions from ventilation to swimming. Both recordings are from the first gill plate flexor, but (A) is at a lower gain than (B). Bar indicates 1 sec.

DISCUSSION

Interpretation of the muscle activity graphs in Figure 2 gives some insight as to the underlying motoneuron activity corresponding to ventilation, hyperventilation, and swimming. If each ventilatory motor burst consists of a nearly equal number of motoneuron discharges, then the observed decrease in burst duration would entail a progressively smaller interspike interval. Assuming that the opisthosomal flexor and extensor muscles undergo summation and facilitation, such a reduction in interspike interval would explain the observed increase in muscle potential amplitude. The amplitude change in the muscle activity with the onset of hyperventilation may result from recruitment of motoneurons causing additional muscular summation and facilitation. The burst shape transition from symmetrical to saw-tooth (Fig. 1c), noted during periods of hyperventilation, may represent

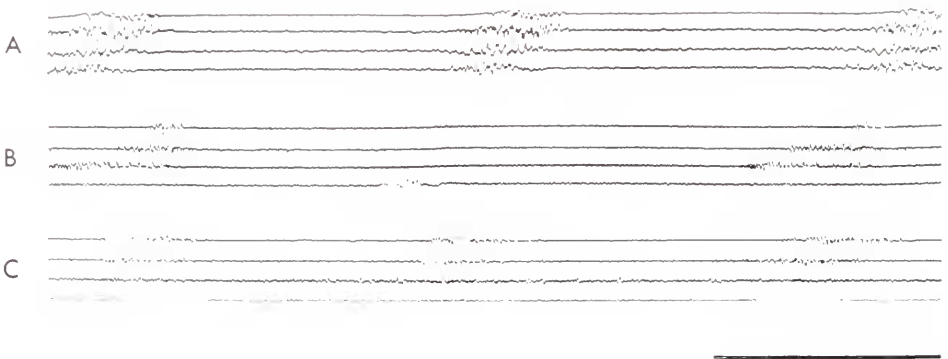


FIGURE 7. The effect of progressive isolation of the opisthosomal ganglia upon ventilatory activity in the flexor muscles of gill plates one through four (in order from top to bottom). The rhythmic, metachronal muscle potentials in (A) are typical of normal ventilatory activity. Desynchronized activity of the fourth gill plate flexor muscles, shown in (B), occurred after the connectives between the third and fourth ganglia were severed. Muscle bursts following the isolation of all opisthosomal ganglia, (C), demonstrate some loose coordination of activity. Bar indicates 1 sec.

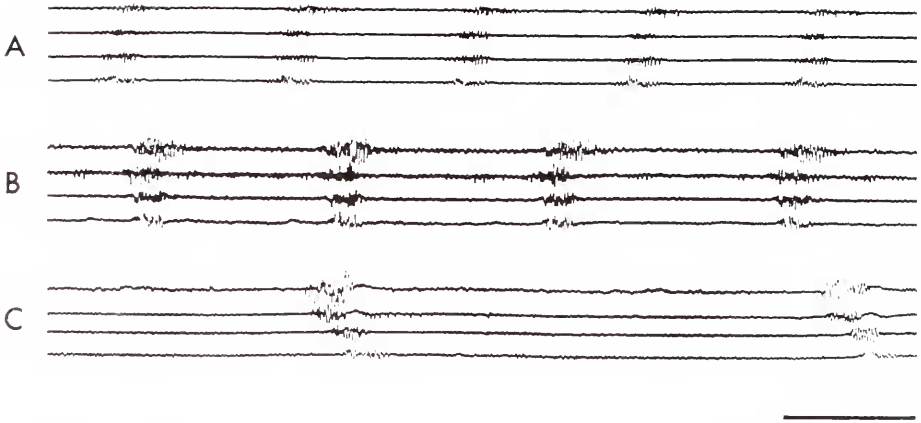


FIGURE 8. Shift in pacemaker location in the intact nervous system following differential stimulation of the leading ganglion as reflected by flexor muscle activity in the first four gill plates (top to bottom). (A) shows the normal metachronal progression of flexor activation. (B) and (C) show the second and first ganglia exercising the pacemaker function. Bar indicates 1 sec.

the migration of these newly recruited motoneuron discharges from the middle to the beginning of the flexor burst. The substantially greater muscle potential amplitude in the flexors and extensors during the performance of swimming movements suggest still further recruitment of motoneurons to the respective motoneuron bursts. The similarities of the burst amplitude - burst interval plots (Fig. 2) and the intersegmental delay - burst interval plots suggest that amplitude and delay are governed by the same principle.

The variability of the pacemaker in the intact nervous system indicates that although the coordinating mechanism for the metachronal rhythm is certainly inherent to the CNS (Fourtner *et al.*, 1971), the directionality of the mechanism is not so stable that it may be considered polarized, as is the case in the crayfish swimmeret control system (Ikeda and Wiersma, 1964). The observation that selective stimulation of any one ganglion triggers ventilatory output in that ganglion and that this activity in turn excites the more remote ganglia both caudally and rostrally in a sequential manner (Fig. 8) intimates that the triggering of these opisthosomal ganglionic oscillators is a threshold phenomenon based on integrated peripheral and central input and that the firing of an oscillator activates interneurons which synapse, either directly or indirectly, upon the more caudal and more rostral oscillators. Although correlated bursting of isolated opisthosomal ganglia may be sustained through sensory input triggering alone (Fig. 7c), burst duration and delay seem to be critically modulated by such interganglionic neural activity possibly in the form of phasic excitation and inhibition transmitted *via* coordinating interneurons analogous to those found in the crayfish swimmeret control system (Stein, 1971). That transection of the connectives to the most caudal ganglion interferes substantially with the control of interganglionic delay and burst duration in the remaining, intact ganglia (Fig. 7b) implies that the

coordinating information emanating from the single ganglion influences the burst generating mechanism in each of the other ganglia.

The initial proposition of a common central control mechanism which mediates both ventilatory and swimming outputs is still not clearly resolved. Judging from the graphs in Figure 2, hyperventilation is most probably an extension of ventilatory activity. The aberration of muscle burst amplitudes and intersegmental delay during swimming suggest the influence of a second control system. Nerve lesion experiments, in which the opisthosomal nervous system is isolated from the brain, seem to confirm this interpretation. However, frequency dependent command fiber tracts in *Limulus* have been reported by Wyse (1971), thus, the apparent loss of swimming movement capabilities in the isolated appendages may be attributed to the elimination of descending command information which coded for a swimming output from the single control system. The swimming and ventilatory coordinating mechanisms are similar, if not one and the same, in that each opisthosomal ganglion is capable of functioning as the pacemaker for both behaviors after lesion of its caudal connectives. Furthermore, the transition from ventilation to swimming can be a gradual progression in terms of muscle activity (Fig. 6a), devoid of observable interaction of two control systems. Since recorded muscle activity is many times removed from the pattern generating event, conclusive evidence in support or repudiation of a single central control mechanism must come from direct central and motor nerve recordings.

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SUMMARY

1. Ventilatory and swimming movements in *Limulus polyphemus* are described and the underlying muscular activity is analyzed.

2. Ventilatory and swimming muscle activity in gill plates consists of repetitive, metachronal bursts in both extensor and flexor muscles beginning in the caudal gill plate muscles and proceeding sequentially forward.

3. Ventilatory muscle bursts are approximately symmetrical, being of relatively long duration, small amplitude and long intersegmental delay, affecting exclusively the gill plate musculature.

4. Hyperventilation involves the active participation of the genital operculum in the rhythmic cycle. Rapidly decreasing intersegmental delay, increasing muscle burst amplitude and transition in the muscle burst shapes accompany decreasing interburst intervals.

5. Swimming movements consist of comparatively high frequency, large amplitude excursions of the gill plates, genital operculum and, to various degrees, the walking legs. Metachronous, high amplitude, square shaped muscle bursts, which proceed rostrally with a short intersegmental delay, are responsible for these propulsive movements.

6. Swimming movements of the gill plates require descending information from the brain, whereas the neural circuitry required for ventilation and hyperventilation is endogenous to the opisthosomal ganglia.

7. The pacemaker function for swimming and ventilatory movements may be served by any of the five involved opisthosomal ganglia if its caudal connectives are severed.

8. The ventilatory coordinating mechanism is not polarized; whichever ganglion functions as the pacemaker, the wave of excitation propagates both rostrally and caudally, sequentially exciting neighboring ganglia.

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